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LETTER TO JMG

Four novel mutations in the *OFD1 (Cxorf5)* gene in Finnish patients with oral-facial-digital syndrome 1

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Oral-facial-digital syndrome type 1 (OFD1, MIM 311200) was first described by Papillon-Léage and Psaume[1] in 1954 and further delineated in 1962 by Gorlin and Psaume,[2] who called it orodigitofacial dysostosis. It is a multiple congenital anomaly syndrome characterised by malformations of the face, oral cavity, and hands and feet. The facial dysmorphic features include hypertelorism, frontal bossing, broad nasal bridge, hypoplasia of alar cartilage, and transient milia. Oral cavity malformations include often asymmetrical cleft of the palate (80%), small midline cleft of the upper lip (45%), clefts of the tongue, hamartomatous masses on the ventral surface of the tongue (70%), mucobuccal fibrous bands, and dental abnormalities. Malformations of the fingers are seen in 50-70% and toe malformations in 25%. Central nervous system abnormalities, such as hydrocephalus, porencephaly, and agenesis of the corpus callosum, with mild mental retardation are seen in 40%.[3] In recent years, a kidney disease closely resembling adult type polycystic kidney disease has been shown to be one of the distinct features of this syndrome.[4 5]

At least nine different forms of oral-facial-digital syndromes have been described, type 1 being the most common with a suggested incidence of 1:50 000 live births. OFD1 syndrome has dominant X linked inheritance with lethality in males. However, a case of Klinefelter syndrome (XXY) with OFD1 has been reported.[6]

By linkage analysis in two kindreds, the locus for OFD1 was mapped to Xp22.3-22.2.[7] Recently, the gene for OFD1, *Cxorf5*, was identified, and mutations of three familial and four sporadic cases were identified by Ferrante *et al*.[8] Expression of the gene was seen in all the tissues affected in the syndrome.

We report here the identification of four novel mutations in the *OFD1* gene together with the clinical findings in four Finnish families, of which two are familial and two sporadic.

PATIENTS AND METHODS

Patients

The patients were ascertained from the Cleft Centre of the Department of Plastic Surgery, Helsinki University Central Hospital, where all patients with cleft lip and/or palate nationwide are treated. In addition, patients were ascertained from the Department of Medical Genetics of The Family Federation of Finland, which serves the whole country, and the Clinical Genetics Unit of Helsinki University Central Hospital, which serves the densely populated south of Finland in clinical genetics. All the patients were examined (fig 1) and their files and hospital records analysed by one of the authors (SA-M).

Mutation analysis

DNA extracted from peripheral EDTA blood of the patients was screened for mutations in the *OFD1* gene using primer sequences kindly provided by Dr Brunella Franco from Telethon Institute of Genetics and Medicine (TIGEM). PCR amplifications of the samples were run through 35 cycles consisting of 40 seconds at 94°C (denaturation), 40 seconds at 55 or 50°C (annealing), and one minute at 72°C (extension) with

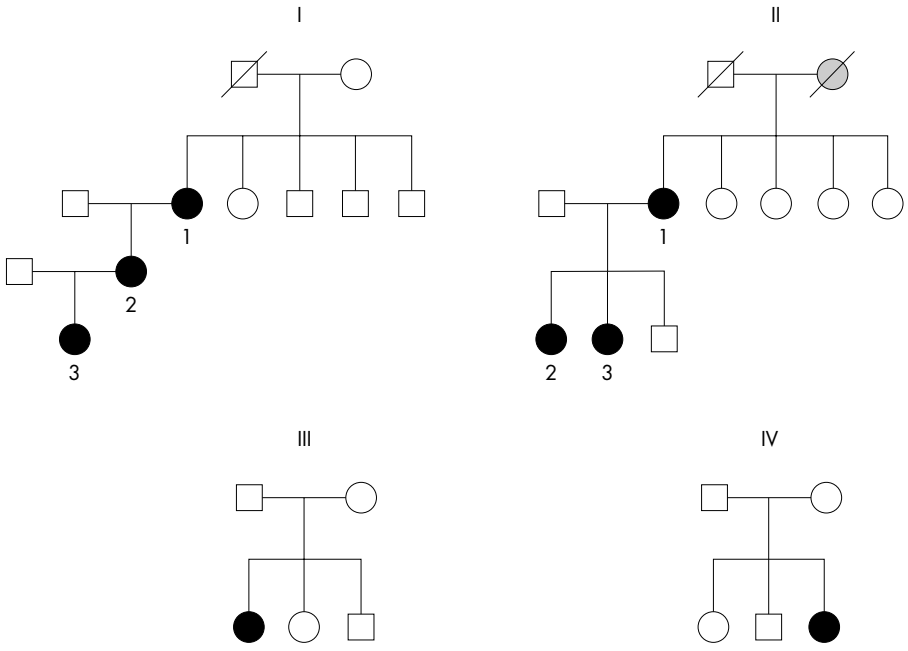

Figure 1 The family pedigrees of the Finnish OFD1 families. Black symbols, affected; symbols with slashed lines, anamnestically affected.

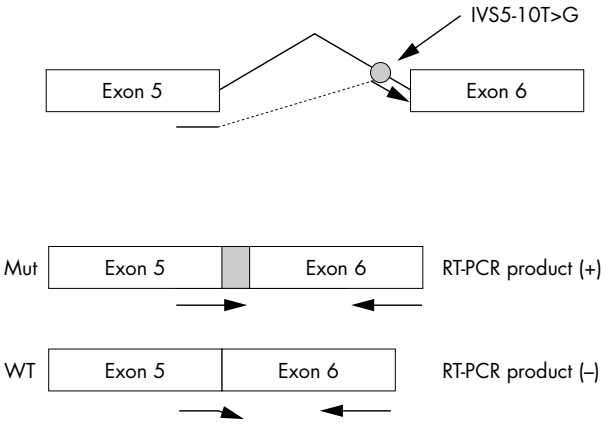

Figure 2 Diagram of detection of the transcript showing the abnormal splicing caused by the IV5-10T>G mutation in exon 6 of the *OFD1* gene.

the final extension step of 5-10 minutes covering all 23 exons. Sequencing of PCR products was performed using ABI PRISM7 BigDye Terminator Cycle Sequencing Kit, Version 2.0 (Applied Biosystems, Foster City, CA, USA) in both directions and analysed using an ABI PRISM7 3100 Genetic Analyzer according to the manufacturer's instructions. The presence of a mutation was confirmed by minisequencing[9] of the DNA in each family member. To exclude the presence of each of the mutations in random subjects, DNA extracted from buffy coat samples of 50 anonymous Finnish blood donors were analysed by minisequencing.

Ethical approval for the study was obtained from the ethical committee of Helsinki University Hospital and the Finnish Red Cross Transfusion Service.

RNA analysis

RNA was isolated from heparin blood samples of the control and the youngest patient from family I (fig 1) carrying the intronic mutation IVS5-10T>G using the QIAamp RNA Blood Mini kit (Qiagen, Hilden, Germany). This mutation generates a putative novel splice site in exon 6. The mRNA was reverse transcribed to cDNA using 1 µg of total RNA, 10 units of AMV reverse transcriptase (Promega M5101) in the presence of 20 units of recombinant RNase inhibitor (RNasin, Promega, N2511), and 25 nmol dNTPs. The reaction was allowed to take place at 42°C for one hour, after which the cDNA was diluted with 1.7 volumes of DNA-TE-Buffer (10 mmol/l Tris-HCl, pH 7.8, 1 mmol/l EDTA) and stored at –20°C. cDNA synthesis was primed with the antisense primer 5′-ACTTGTCTGAGTTTCCATATTACAACTC-3′ located in the coding sequence of exon 6 of the *OFD1* mRNA. For PCR two sense primers were designed. The first one, 5′-CATTAAAATCAACCCTACTTCCAGTCTC-3′, located in exon 4, together with the reverse primer used in the reverse transcription flanked the putative new splice site. The second sense primer 5′-AGGATCTGATAAAGAAAATCAAAAAGGTTT TTTAGGTTT-3′ was designed to anneal exclusively over the putative novel splice site to give a product only if this putative new splice site was transcribed (fig 2).

RESULTS

We found four novel mutations in the *OFD1* gene (table 1, fig 3) in two sporadic patients and in two families, both containing three patients with OFD1 syndrome (fig 1). The clinical features of the patients shown in table 2 were characteristic of OFD1 syndrome. In each case a novel mutation in the recently discovered *OFD1* gene was identified; two of them were frameshifts, one was a missense mutation, and one was a splice mutation.

Table 1 Mutations in patients with OFD1

Family (case*)	Location	Nucleotide change†	Effect on protein
I (F)	Intron 5	IVS5-10T>G	Abnormal splicing
II (F)	Exon 16	1887-1888insAT	Frameshift
III (S)	Exon 3	235G>A	A79T†
IV (S)	Exon 13	1409delA	Frameshift

*F=familiar, S=sporadic.
†Mutation description is according to Antonarakis *et al*[12] with the cDNA sequence of *OFD1* used as the reference and with the ATG translation initiation codon denoted as nucleotide +1.

In family I, the syndrome was diagnosed in three successive generations (fig 1). The grandmother's facial features were typical of OFD1. She did not have cleft palate like her daughter and granddaughter. Instead, alveolar notching with missing teeth were seen. No abnormalities of the hands were seen. At the age of 44 years, she had just undergone a kidney transplant because of polycystic kidney disease. The kidney disease had been discovered by chance on routine gynaecological examination one year earlier and dialysis treatment was started almost immediately after that. She was unwilling to participate in genetic DNA studies. The daughter had small hands and feet with brachydactyly of the fifth fingers. The syndactyly of her fourth and fifth fingers of the left hand had been operated on as a child. Renal ultrasonography was performed at the age of 23, when the diagnosis of OFD1 was confirmed. Multiple cysts were seen in the right kidney, but no signs of renal failure in the laboratory examinations was found. The granddaughter, aged 1.5 years, has developed normally. In the extremities, there was only mild clinodactyly of the fifth fingers. The cleft palate was asymmetrical. Alveolar notching, suggesting tooth aplasia, and mucobuccal fibrous bands were seen. No signs of retardation were detected in this family. We found a T>G change in intron 5 of the *OFD1* gene in the daughter and the granddaughter. The mutation is located 10 nucleotides before the starting nucleotide of exon 6 (fig 3) where it creates a novel splice acceptor site (and adds three novel amino acids to the 5′ end of exon 6) resulting in an alternative splicing of mRNA. This was confirmed by the RNA studies described in the Methods section (fig 4).

In family II (fig 1), the mother and her two daughters were clinically examined and their facial features and other signs were typical of OFD1 syndrome (table 2). All three patients studied had midline pseudocleft of the upper lip, but no operations had been performed. The tongues of the mother and the older daughter were bilobulated and the younger daughter had multiple lobules in her tongue. No-one in this family had had problems with kidney function and no ultrasonographic examinations of the kidneys were performed. At the age of 42 years, the mother was diagnosed with hyperthyreosis, which was treated with radioactive iodine. The younger daughter had been operated on at the age of 1 year because of a medially located, supernumerary distal phalanx in the right hallux. The left leg grew 3 cm longer than the right leg and at the age of 13 years an orthopaedic operation was performed. The left breast has grown bigger than the right with mastopathic changes. Her mental development has been mildly delayed and she attended a special school. In the older daughter, vaginal bleeding started at the age of 3 months. After investigations, hormonal medication was given for precocious puberty. Epileptic seizures began at the age of 2½ years. Repeated CT scan of the brain showed a hypothalamic hamartoma, which was thought to be the reason for the precocious puberty through excretion of hypothalamic hormones. She had short stature with a final height of 1.45 m (–3.5 SD) and small hands and feet. The fourth metatarsals were short, especially in the right foot. She attended a

Table 2 Clinical features of the patients with OFD1

	I.3	I.2	IV	III	II.3	II.2	II.1
Age (years)	1.5	23	0.5	30	19	25	50
Clinical findings							
Facial							
Midfacial flattening	+	+	+	+	–	–	+
Alar hypoplasia	+	+	+	+	+	+	+
Dystopia canthorum	+	+	+	–	+	–	+
Skin milia	+	–	+	–	–	–	–
Oral							
Thin upper lip	+	+	+	+	+	+	+
Cleft palate	+	+	–	–	–	–	–
Midline pseudocleft of upper lip	–	–	–	–	+	+	+
Alveolar notching	+	+	+	+	–	+	+
Tooth aplasia	NA	+	NA	+	+	+	+
Lobulated tongue	+	+	+	+	+	+	+
Tongue hamartoma	+	+	+	–	+	+	–
Multiple frenula	+	+	+	+	–	+	–
Cerebral							
Mental retardation	–	–	–	–	+	+	–
Renal							
Polycystic kidneys	ND	+	ND	+	ND	ND	ND
Extremities	+	+	+	+	+	+	+

NA=not yet available, ND=not done.

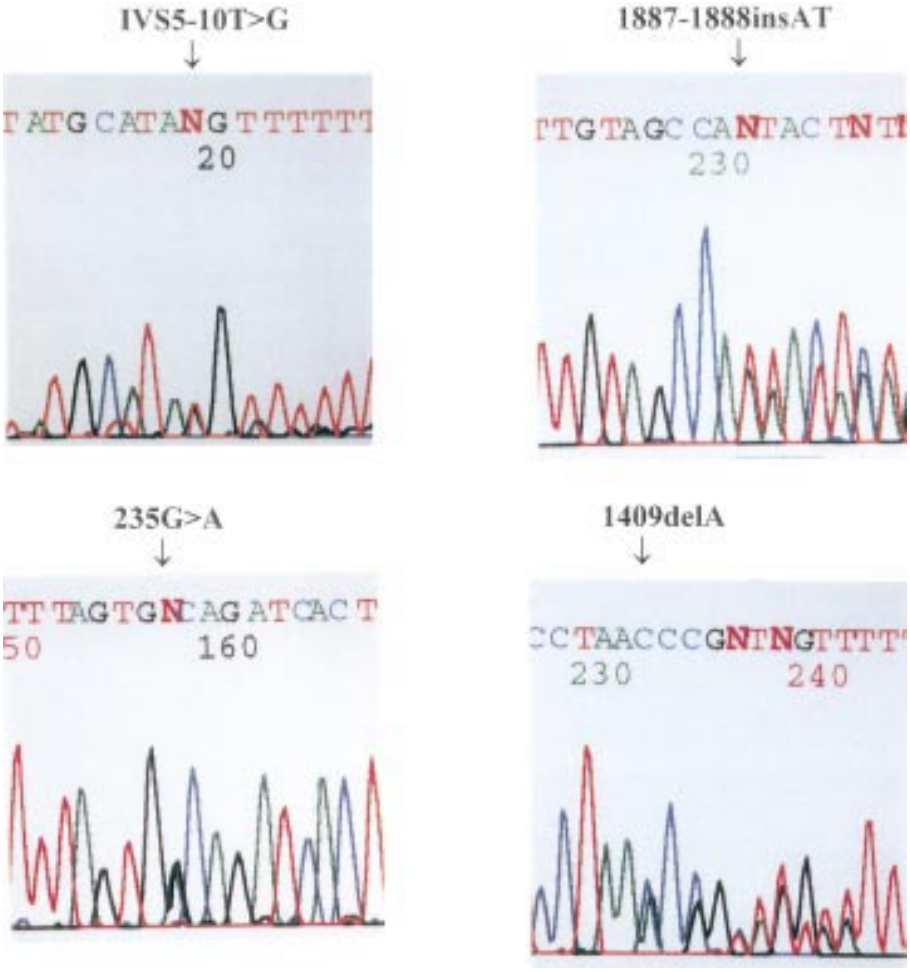

Figure 3 Sequencing chromatograms showing the four *OFD1* mutations in the Finnish patients.

special school for handicapped children because of moderate mental retardation and received medication for psychiatric symptoms for a couple of years. In this family, an insertion of AT between nucleotides 1887 and 1888 in exon 16 was detected in all three family members (fig 3). This creates a frameshift resulting in a premature stop codon (TAG) at amino acid position 666 of the *OFD1* gene.

In family III, the only patient studied had syndactyly of the fourth and fifth fingers of the left hand that had been operated on at the ages of 5 and 11 years. On ultrasonographic

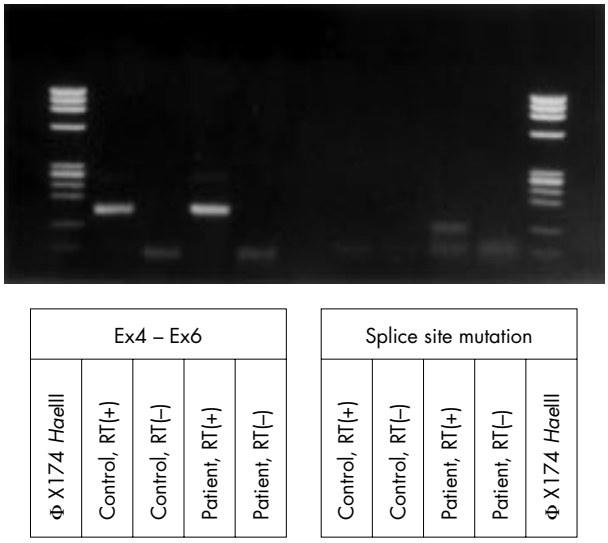

Figure 4 The RT-PCR-products covering exons 4-6 are normal in both control and patient samples (on the left). The intronic nucleotide change IVS5-10T>G results in an abnormally spliced product in the patient sample (RT(+)) compared to the normal sample (RT(+)). RT(–) samples are the control samples with no cDNA.

examination, numerous small cysts were detected in both kidneys at the age of 29 years. Functional studies of the kidneys were normal. In this patient a missense mutation G>A at nucleotide 235 in exon 3 was identified (table 1, fig 3). This transversion leads to a change of a non-polar amino acid alanine (A) to an uncharged polar amino acid threonine (T). We analysed DNA samples from both parents by minisequencing and no abnormalities were found, indicating that this is a de novo mutation.

In family IV, the index case was first examined at the age of 6 months. The first diagnostic signs were a prominent metopic ridge and a soft nodule (about 0.5 cm in diameter) medially in the right hallux. Psychomotor development has proceeded within normal limits. In this patient, a deletion of A at nucleotide 1409 in exon 13 leading to a frameshift was identified. This mutation results in a premature stop codon (TAG) at position 472. DNA from both parents was analysed and no mutations were found.

None of the four mutations was identified in the DNA of 50 anonymous Finnish blood donors screened by minisequencing.

RNA

The results of the RT-PCR experiments (fig 4) show that in both the patient and the control sample the products generated by RT-PCR amplifying the area flanking the putative novel splice site are of similar size, indicating that the normal sized mRNA could be found in both samples. However, the splice site specific RT-PCR resulted in the identification of the product only in the patient's sample. This indicates that the intronic nucleotide change T >G residing 10 nucleotides from the splice acceptor site of exon 6 generates a false splice site and so is most likely the cause of the disease in this patient.

DISCUSSION

Eight OFD1 patients have been diagnosed in Finland, consisting of a population of about 5 million, during the last 20 years. In all of them, a mutation in the recently identified *OFD1 (Cxorf5)* gene was found. Two of them were nonsense, one missense, and one splice mutation. The clinical features were characteristic in every patient. Interestingly, one of our patients had short fourth metatarsals, similar to a patient described by Ferrante *et al*.[8] Mild or moderate mental retardation was seen in one of the families with the two daughters with learning difficulties.

- Oral-facial-digital syndrome type 1 (OFD1) is an X linked dominant disorder characterised by malformations in the face, oral cavity, and digits with a wide phenotypic variation. Recently, mutations in the *OFD1* gene (*Cxorf5*) at Xp22 were found to underlie OFD1. We report here the identification of four novel mutations in the *OFD1* gene in the Finnish families, two of which are familial and two sporadic.
- In the familial cases a splice mutation T>G in intron 5 in the mother and her daughter was identified resulting in an abnormal splicing, and in the second family a nonsense mutation 1887-1888insAT in exon 16 was detected in the mother and her two daughters. Analysis of the sporadic cases showed a missense mutation 235G>A in exon 3 and a single nucleotide deletion 1409delA leading to a nonsense mutation in exon 13. Three of the mutations in this study were located in the same exons as in the original study.
- Our study confirms the causative role of the *OFD1* gene in the pathogenesis of oral-facial-digital syndrome type 1.

Renal involvement in OFD1 cases may be as high as 50%.[10] In three out of eight Finnish patients, polycystic kidney disease was present, and one of them received a new kidney at the age of 44 years. The mutations that were associated with polycystic kidney disease in the Finnish patients were the splice mutation in intron 5 and a missense mutation G>A at nucleotide 235 in exon 3. In the original report by Ferrante *et al*,[8] polycystic kidney disease was also associated with mutations in exon 3 but also in intron 4. Polycystic kidney disease usually manifests in adulthood, so two of our patients are too young to be able to draw any conclusions about kidney disease.

When analysing the phenotype-genotype correlation concerning mental retardation associated with this syndrome, mild to moderate mental retardation or learning difficulties were reported with mutations in exons 3, 13, and 16, and intron 4 in the original study.[8] In this study, only the frameshift mutation in exon 16 was associated with learning difficulties in two out of three members of the same family. Further studies are needed to know whether certain mutations are more frequently associated with kidney disease or mental retardation, the findings that are important in genetic counselling when predicting the outcome of the disease.

The *OFD1* gene contains 23 coding exons (GenBank accession numbers Y15164 and Y16355) with unknown function.[11] Interestingly, three of the mutations found in this study are located in the same exons 3, 13, and 16 as the mutations reported in the original study by Ferrante *et al*,[8] suggesting that these exons might represent regions for mutational hot spots. Functional studies of both the wild type *OFD1* gene and the mutants are needed to understand the disease mechanism underlying OFD1.

In conclusion, we report here the identification of four novel mutations in the *OFD1* gene in seven Finnish patients with oral-facial-digital syndrome type I. Our results confirm the causative role of the *OFD1* gene in the pathogenesis of this syndrome.

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ECHO

Genes predict outcome in multiple sclerosis

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Pairs of siblings with multiple sclerosis show the same progression of their disease and the same eventual disability and handicap, supporting the theory that genes rather than environment dictate both susceptibility to multiple sclerosis and its outcome.

This is the conclusion of Chataway *et al*, who have added to the first UK cohort of 177 sibling pairs with multiple sclerosis from 166 families and reanalysed the data for the new total of 262 pairs from 250 families. As before, they looked for concordance in clinical variables in each pair of siblings for course of disease, presenting symptoms, age and year of onset—and this time also included measures of disability, disease progression, and handicap. The data were adjusted for confounding factors associated with analysis of sibling pairs and were analysed with statistical techniques that can include potentially confounding variables.

A third of all sibling pairs had similar presenting symptoms, but this was not statistically significant, nor was the primary affected site. However, 50% of the sibling pairs had an identical course of their multiple sclerosis—relapse-remitting, primary progressive, or secondary progressive—which was a significant result. Severity of the disease at assessment indicated that disability, progression, and handicap were concordant within sibling pairs but relapse rate in the previous year was not.

So although most members of sibling pairs have different initial symptoms, the progress of their disease will converge such that each sibling in a pair will eventually have similar disability and handicap scores.

▲ *Journal of Neurology Neurosurgery and Psychiatry* 2001;**71**:757–761.